

CRAEMS: Molecular Environmental Chemistry of Mn Oxide Biomineralization (CHE-0089208)

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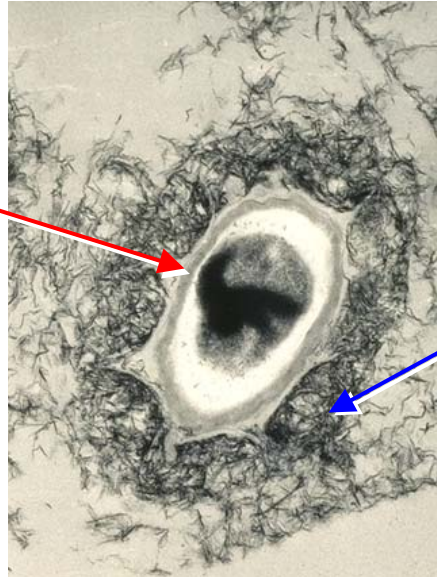
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Mn(II)-oxidizing Bacteria:

- Ubiquitous in fresh, marine waters.
- Primary source of environmental Mn oxides.



Biogenic Mn-oxides:

- Reactive!
- High surface area.
- Scavenge heavy metals.
- Degrade organics.

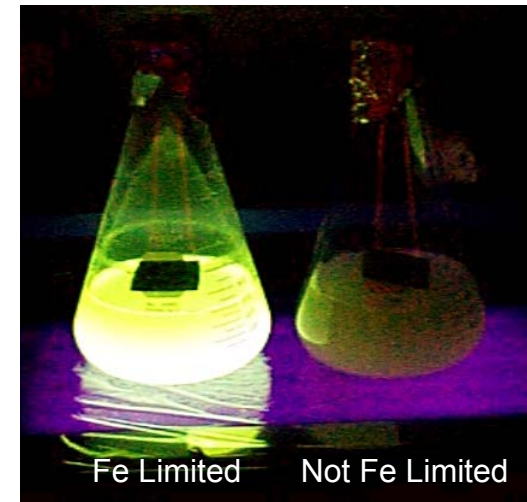
Key Questions:

- How do bacteria oxidize Mn(II)?
- What are the identities of biogenic Mn oxides?
- How do they react with aqueous solutes?

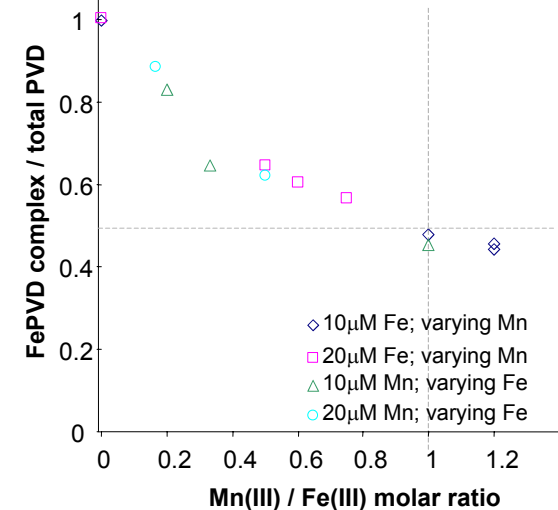
✉ *Key controls on contaminant and nutrient cycling in the environment.*

A siderophore from a Mn(II)-oxidizing bacterium complexes Mn(III)

- Pyoverdine (Pvd) is a class of fluorescent siderophores found in many *Pseudomonas* species
- Binding of Pvd to Fe(III) or Mn(III) decrease fluorescence similarly.
- The Mn(II)-oxidizing *Pseudomonas putida* strains GB-1 and MnB1 make a Mn(III)-Pvd complex which is stable against precipitation or disproportionation into Mn(II) and Mn(IV) compounds. This complex is robust against metal exchange with Fe(III) or ligand exchange with pyrophosphate.
- Environmental implications: Mn(III)-siderophore complexes may:
 - contribute to the pool of soluble Mn
 - serve as effective environmental oxidants
 - confer a competitive advantage in Fe(III) scavenging to siderophore-producing Mn-oxidizers over other siderophore producers that cannot oxidize Mn

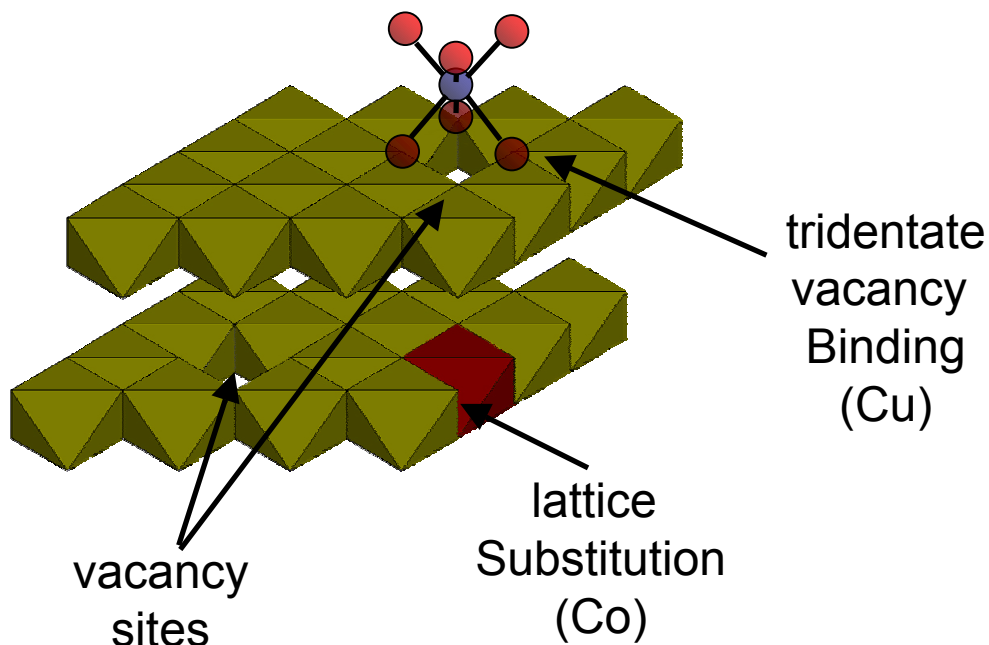


Pyoverdine fluorescence as observed when exposed to a UV light source



Simultaneous addition of Mn(III) and Fe(III) with 1 mM Na citrate in 67 mM HEPES buffer, pH 8, PVD=8 μM

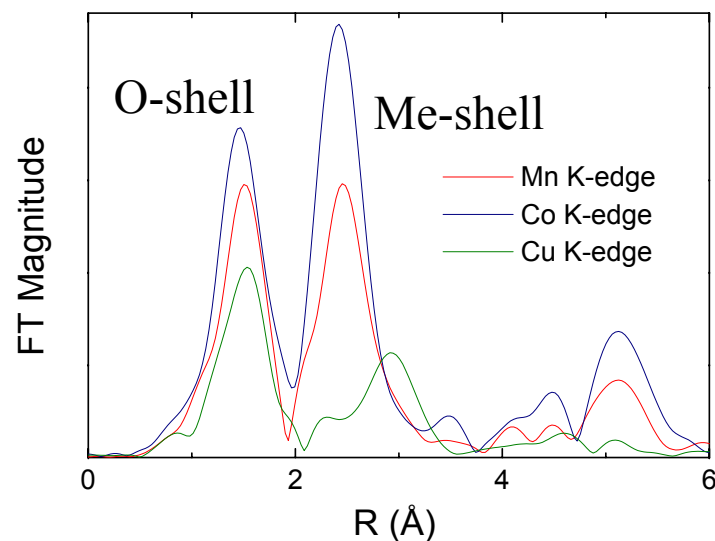
How are Heavy Metal Contaminants Sequestered into Biogenic Manganese Oxides?



EXAFS spectroscopy shows that the incorporation of Cu and Co differs significantly. Co(II) is oxidized by the Mn oxide and the resultant Co(III) is substituted into the layer structure. Cu(II) absorbs mostly in tridentate vacancy sites, with little incorporation within the manganate layers.

Why should you care about this?

- Heavy metals, such as cobalt and copper, threaten potable water supplies throughout the West (Pinal Creek, Arizona).
- Manganese oxides are excellent adsorbents for cobalt and copper.
- Biogenic manganese oxides may provide natural attenuation.



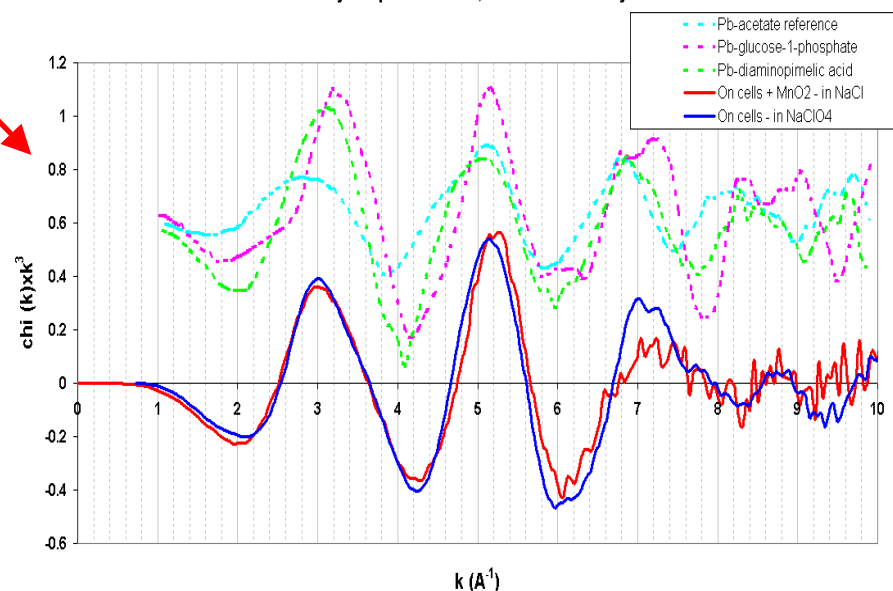
L3-edge Pb EXAFS on *Pseudomonas p.* Mn Oxide Systems
1-2 days equilibration, 0.01 M NaClO₄

Pb(II) Equilibration on *Pseudomonas putida* Mn oxide cell systems EXAFS results - 1

Aqueous Pb(II) reacts with either the organic -possibly phosphated- groups of probable bacterial cell origin, or with the biogenic Mn oxide, depending on the electrolyte anion used for equilibration.

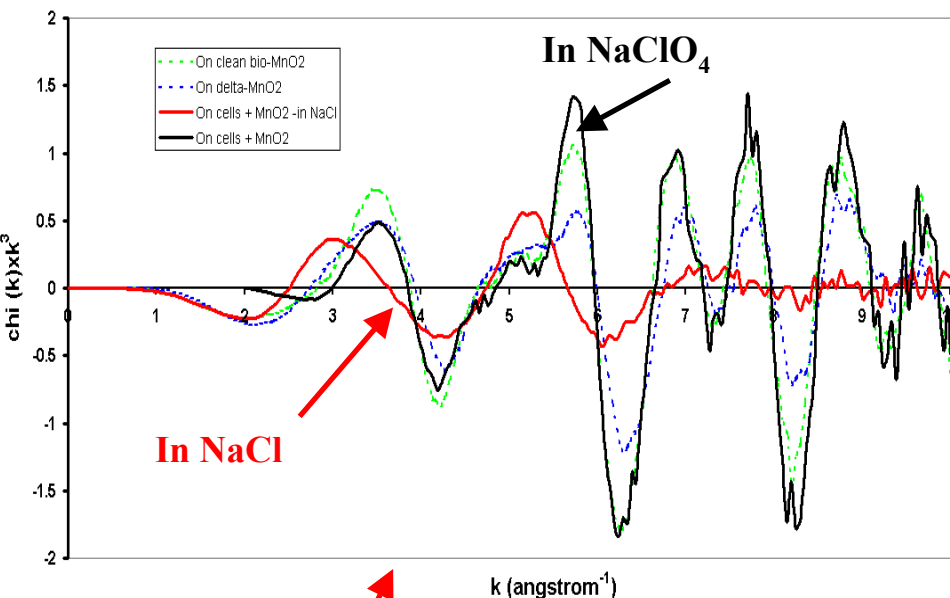
M. Villalobos, B. Toner, and G. Sposito, UCB
J. Bargar (SSRL)

L3-edge Pb EXAFS on *Pseudomonas p.* Cell Systems
1-2 days equilibration, 0.01 M electrolyte

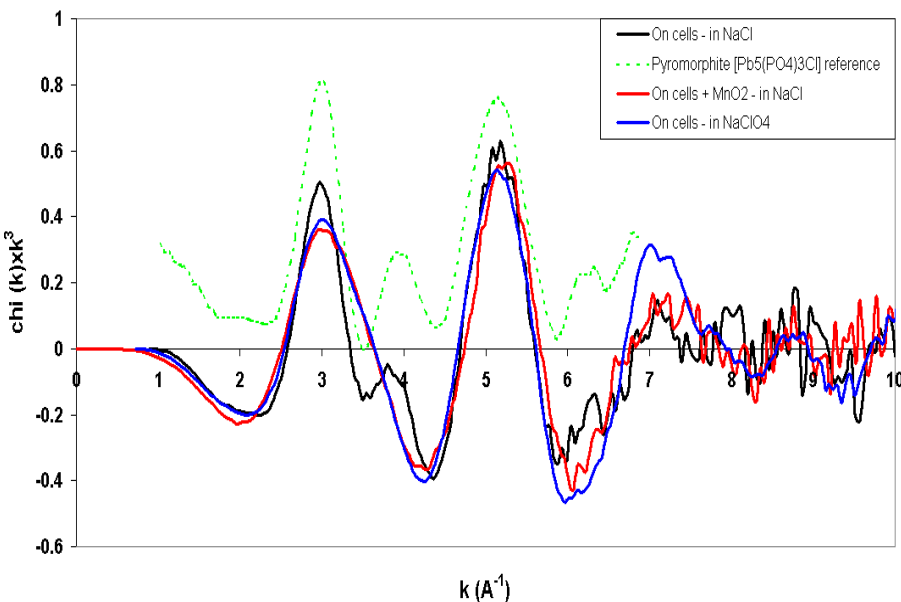


Cl⁻ ions stabilize Pb(II) complexation to organic components, probably via formation of mixed Cl⁻-phosphate complexes. Therefore, Cl⁻ shifts the affinity of Pb from the Mn-oxide to organic groups, possibly of cell origin where the most probable source of phosphate is found.

This result points to the importance of the experimental conditions for equilibration, especially because Cl⁻ is a more relevant environmental ion than ClO₄⁻.



L3-edge Pb EXAFS on *Pseudomonas p.* Cell Systems
1-2 days equilibration, 0.01 M electrolyte



Pb(II) Equilibration on *Pseudomonas putida* Mn oxide cell systems EXAFS results - 2

In the limiting case of Mn oxide absence, Pb(II) ions form a pyromorphite-like phase.

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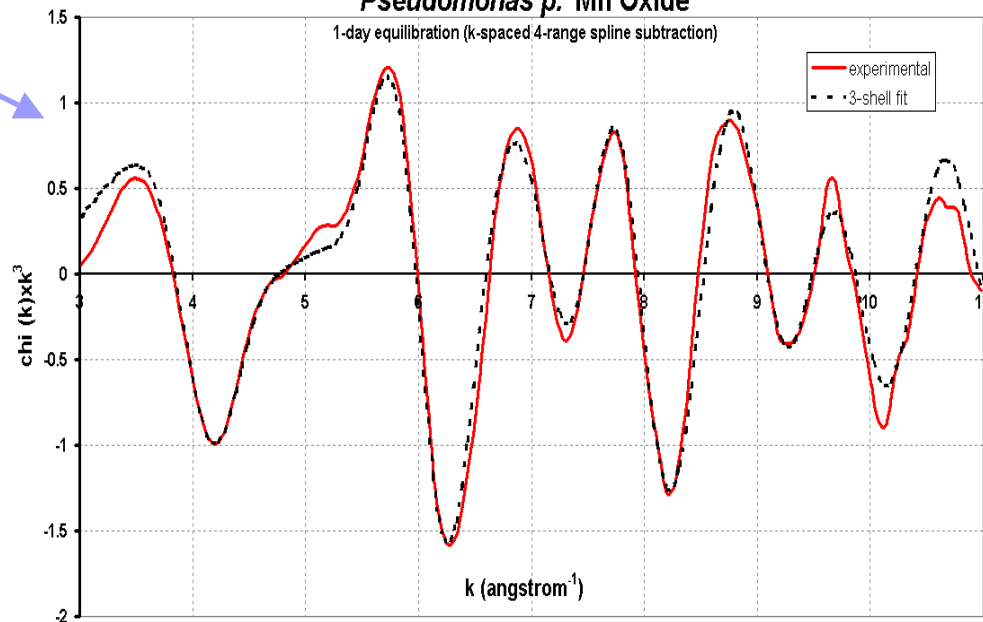
In the absence of Cl^- (or of cell components) Pb (II) reacts with the biogenic Mn oxide and is incorporated into its structure.

The complex formed on this oxide occupies the interlayers above and below vacant sites, forming a tridentate triple-corner sharing complex, similar to Zn in calcophanite (a Zn-Mn layered oxide).

No edge-sharing or face-sharing complexes were detected.

This applies to all synthetic layered Mn(IV) oxides investigated as well.

Pb L3-edge EXAFS of 1.06 mmol Pb/g *Pseudomonas p.* Mn Oxide



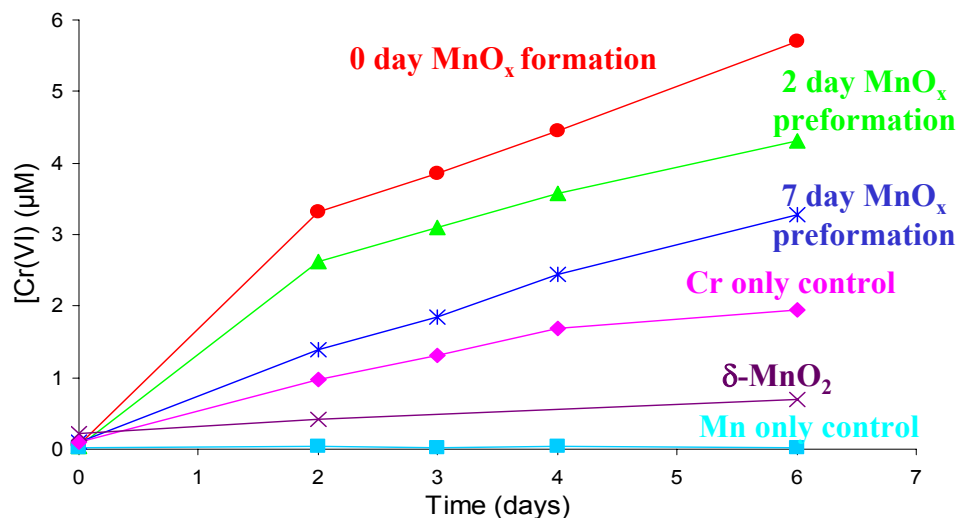
Mn(II)-oxidizing spores of *Bacillus* sp. SG-1 oxidize Cr(III) faster than biogenic or synthetic Mn(IV) oxides

Research Questions

How do rates of oxidation of Cr(III) by biogenic Mn oxides compare with synthetic Mn oxides?

Does the Mn(III) intermediate in bacterial Mn oxidation increase rates of Cr(III) oxidation?

Is there direct biological Cr(III) oxidation?

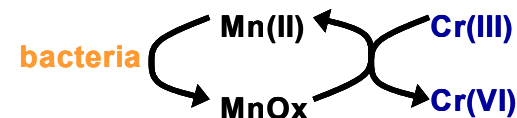


Cr(VI) production during the oxidation of Cr(OH)₃ by SG-1, Mn oxides preformed on SG-1 spores, and δ-MnO₂

Hypothesis: SG-1 spores allowed to form Mn-oxides before the addition of Cr(III) would oxidize Cr(III) faster

Results: The opposite was observed—highest rates of Cr oxidation occurred when Mn-oxides were not present

– Extremely reactive oxide



– Mn(III) intermediate



– Enzymatic Cr(III) oxidation

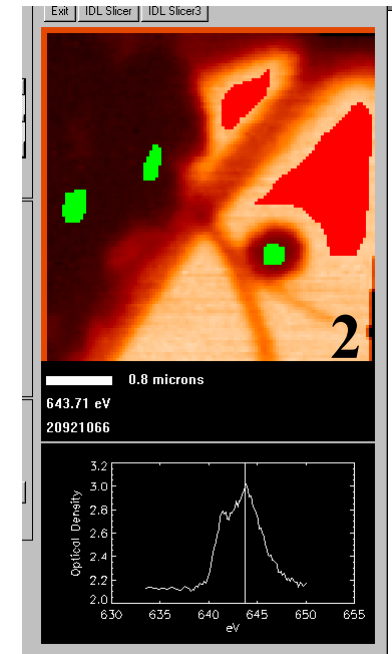
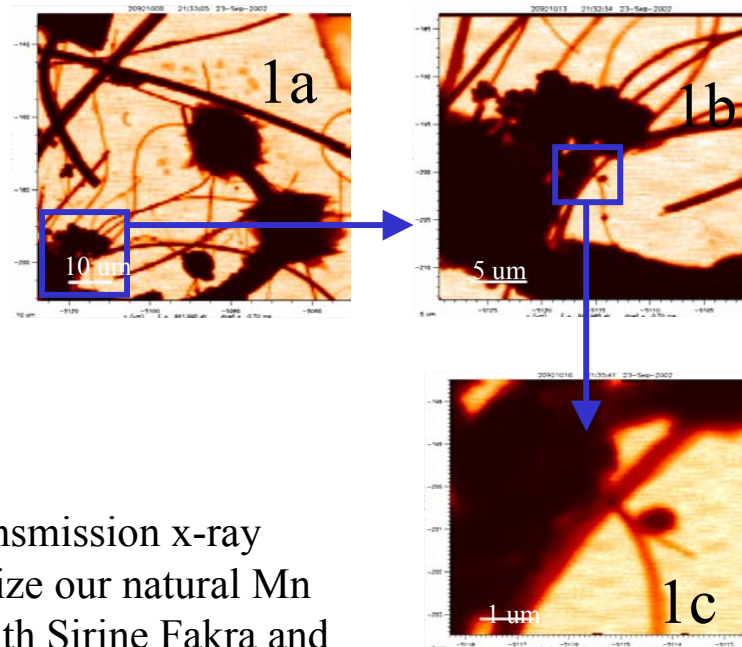


Possible mechanisms for accelerated Cr(III) oxidation by Mn(II)-oxidizing bacteria

Scanning Transmission X-ray Microscopy (STXM) Analysis of Natural Mn Oxides

We are interested in understanding the Mn cycle at the atomic, molecular, and nanometer to kilometer spatial scales. We employ a wide variety **microscopic** and **spectroscopic** techniques to study biogenic Mn oxides. The images and spectrum presented here are of samples taken from the coast of the Black Sea.

We have been using scanning transmission x-ray microscopy (STXM) to characterize our natural Mn oxide samples in collaboration with Sirine Fakra and Tony Warwick at the Advanced Light Source (Lawrence Berkeley National Lab). We are able to acquire microscopic images of hydrated and **undisturbed natural samples** (Figures 1a, 1b, and 1c). We can also measure the oxidation state of Mn in specific places on these samples (Figure 2). When compared to reference materials we are able to determine that the Mn in the green areas is Mn(IV) and looks very much like a birnessite and biogenic Mn oxides produced in the laboratory.



■ I region selected
■ I₀ region selected

Optical density= absorbance= $-\ln(I/I_0)$

Some heavy metals stimulate Mn(II) Oxidation by *Pseudomonas putida* strain GB-1

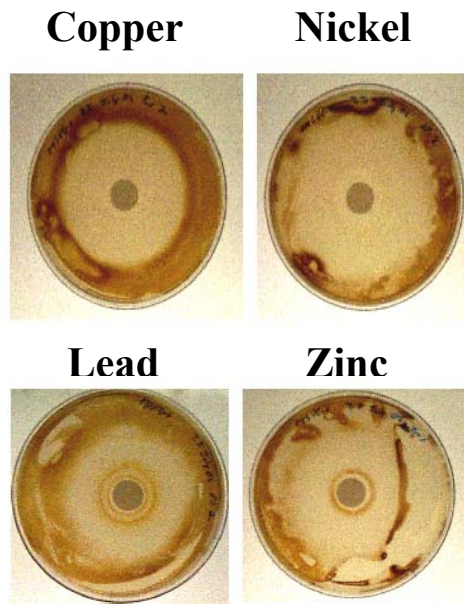
Manganese oxides adsorb and immobilize a variety of heavy metals. Bacteria that oxidize Mn(II) may be protected from the toxic effects of such metals, as the Mn oxides prevent cellular uptake of the heavy metals. Do these bacteria “turn on” or upregulate Mn oxidation in response to toxic metal stress? Preliminary results suggest they do.

We’ve tested the effects of copper, cobalt, lead, zinc, and nickel, and have observed signs of potential upregulation in response to zinc and lead. In the photographs below, orange/brown Mn oxides are produced when bacteria “turn on” Mn oxidation.

Bacterial Manganese Oxidation on Metal-Spotted Agar Plates

On the agar plates shown here, reduced Mn(II) and bacteria have been evenly spread. Heavy metals are spotted onto the central disc, and diffuse into the agar, creating a concentration gradient.

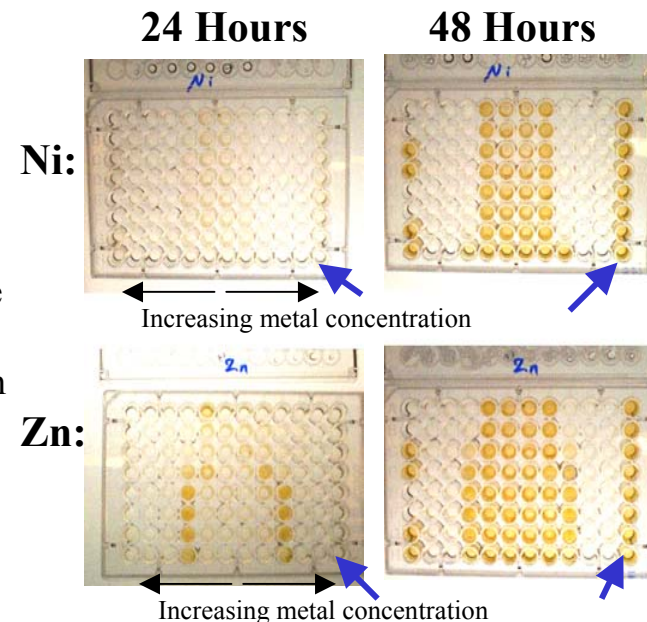
Rings of Mn oxidation close to the central disc on the lead and zinc plates indicate the metal stress at particular concentrations may cue the bacteria to oxidize manganese.



Bacterial Manganese Oxidation in Liquid Media Wells

In the 96 well plates shown here, bacteria and reduced Mn(II) are constant, while nickel or zinc vary across a wide range of concentrations.

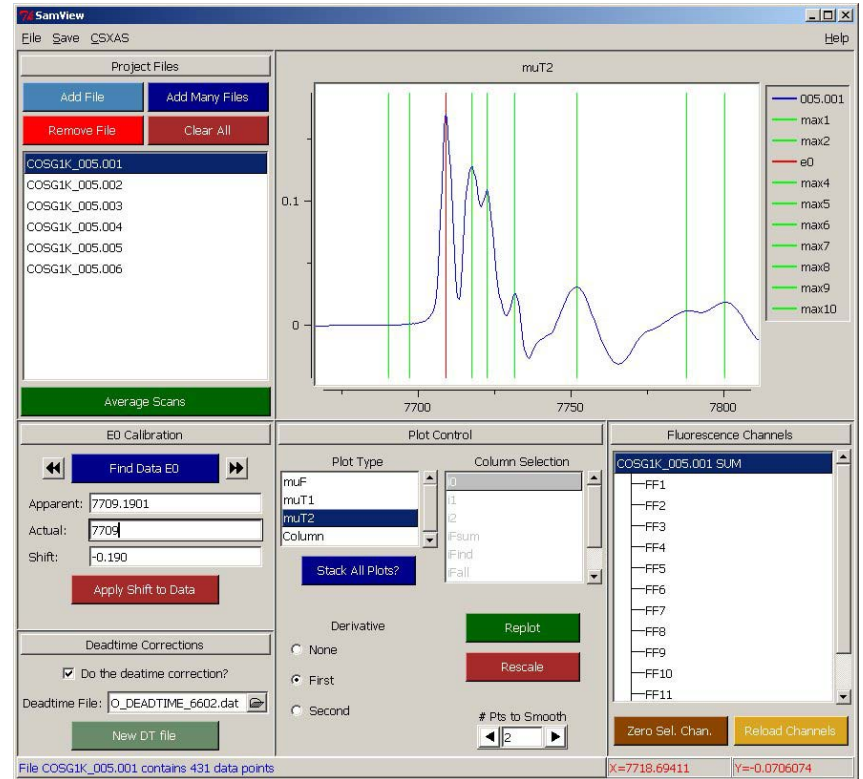
The right column in each plate contains no Zn or Ni. Note that in the Zn plate, Mn oxidation appeared in certain Zn-containing wells 24 hours prior to its appearance in the control, no-zinc wells (marked with a blue arrow).



SixPack

(<http://ssrl.slac.stanford.edu/~swebb/sixpack.htm>)

- All-purpose XAS analysis package developed as part of collaboration
- Features:
 - Data averaging and calibration
 - Accepts data from all US synchrotron sources
 - Background subtraction
 - Principal component analysis
 - Least Squares fitting
 - Contains a fluorescence self-absorption algorithm immensely applicable for environmental and geochemical samples.
 - EXAFS fitting w/FEFF
 - FEFF path creator



- Usage:
 - Obtaining widespread use in the community
 - Over 1250 hits on SIXPack home page since August 2002!